For research use only

E and LE-Series

USER MANUAL

an automated, batch system for
high performance sample preparation
using adaptive focused acoustic energy

Product Name: E/LE-series running SonoLab 6.2.4 or later

Product Number: E220, E220x, LE220, E220R, LE220R

UNIVERSAL PRECAUTIONS

Universal Precautions should be followed on all specimen samples, regardless of whether a sample is known to contain an infectious agent. Laboratories handling specimen samples are advised to comply with applicable parts of the following governmental and clinical standards, or their equivalent in the country of use:

- Centers for Disease Control (CDC), Universal Precautions for Prevention of Transmission of HIV and Other Bloodborne Infections, published 1987, updated 1996
- Clinical and Laboratory Standards Institute (CLSI), GP17-A2 Clinical Laboratory Safety; Approved Guideline - Second Edition, published 2004, ISBN 1-56238-530-5
- Clinical and Laboratory Standards Institute (CLSI), M29-A3 Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline, Third Edition, published 2005, ISBN 1-56238-5674
- Occupational Safety and Health Administration (OSHA), 29 CFR 1910.1030 Bloodborne Pathogens
- International Standards Organization (ISO) 15190:2003, Medical Laboratories – Requirements for Safety

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Covaris products are covered by Patent No. US 6,719,449, US 6,948,843, and other pending applications

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Printed in U.S.A.

Warnings

For safety of operating personnel:

1. Make sure that the equipment is properly grounded. DO NOT operate if it is not properly grounded.
2. The unit is equipped with a power plug appropriate for the destination country. DO NOT, under any circumstances, remove the grounding prong from the power cord.
3. DO NOT attempt to operate the equipment without the safety door closed. When the safety door is open, the mechanical positioning system and the acoustic wave system will not run. If there is any indication that the safety system is not functioning properly, DO NOT operate the equipment and contact Covaris immediately.
4. If the equipment is used in a manner not specified by the manufacturer, the protection provided by the equipment may be impaired.

To prevent damage to the equipment:

1. The instrument is designed to operate in ambient laboratory conditions e.g., 15 to 32°C (60 to 90°F). DO NOT operate the instrument in a cold room environment; the system is designed to operate with a water bath and re-circulating heater/chiller apparatus to control sample temperatures.
2. NEVER run a method without a water bath; this could damage the transducer. The instrument is equipped with a water level sensor to protect the transducer. The system will not allow the acoustic wave treatment to start unless an adequate volume of water is present.
3. ONLY distilled or deionized water should be used to fill the water bath.
4. Empty the water bath and wipe dry EVERY day. DO NOT leave water in the tank for extended time as there is no water filtration or water cleaning system with the apparatus. Drain the degassing lines (see section 4.5 Shutdown Procedure).
5. Establish a standard of operation and periodically test equipment, as described in the Maintenance section of this manual.
6. Be advised that loading third party software onto the computer may interfere with the performance of the Covaris SonoLab™ software. Please consult Covaris.

Warranty

When used in accordance with written instruction and under normal operating conditions, the Covaris instruments are guaranteed to be free of defects in MATERIAL and WORKMANSHIP for one (1) year from the date of original delivery. Any component which proves defective during the stated period will be repaired free of charge or replaced at the sole discretion of Covaris, F.O.B., Woburn, Massachusetts, provided the defective component is returned properly packaged with all transportation charges prepaid. The customer is expected to perform basic diagnostics and component replacement with telephone support from Covaris personnel. If Covaris personnel are required to perform on-site repair, all travel costs are paid by the customer. A limited warranty as specified may apply to certain components of the equipment.

Warranty Exceptions

This warranty is void if failure of the software or hardware has resulted from accidents, abuse, improper maintenance, or repair, or misapplication by the customer. It is also void if damage is caused by any unauthorized attachments or if modifications are made to the equipment. Removing or tampering with the Safety Enclosure will void the warranty, and the customer will assume all liabilities.

This warranty is limited to the original purchaser and is not transferable.

The software will perform according to the accompanying written materials and the medium on which the software is delivered is free of defects in materials under normal use and service. The warranty is void if damage has resulted from third party software not intended for use with the system.

The high power focused transducer is designed to give maximal mechanical energy output in water. Permanent damage to the transducer and electronic circuits could result if the transducer is operated without water. Operation of the system without water in the water bath voids the warranty.

CONTACT COVARIS, INC. SHOULD YOU HAVE ANY QUESTIONS CONCERNING EQUIPMENT.

Warranty Services

The purchased equipment is covered by a twelve (12) month warranty which includes all the service and support necessary so that the customer can operate the equipment successfully. Extended warranties are available at the end of the original 12 month warranty period.

Services included with the original purchase of the system are:

Installation and Training – Setup and installation of the equipment and operator training can be purchased at time of initial acquisition of the equipment. Both will be performed by a qualified Covaris service representative. One half day will be scheduled to perform the installation and on-site training.

- The operators' training will include valuable hands-on time with the equipment.
- Preventative maintenance and troubleshooting tips will also be covered.

Technical Support – On-going assistance with the operation or application of the equipment and/or troubleshooting is provided via:

- Telephone (+1 781 932 3959) during the hours of 9:00am to 4:00pm, Monday through Friday,
    - United States, Eastern Standard Time (EST).
    - Greenwich Mean Time (GMT-05:00)
- E-mail queries to techsupport@covarisinc.com

Parts Replacement – Replacement of parts (excluding consumables and cables) for normal operation of equipment are provided on a priority basis. Shipping charges are included. Failure due to accident, abuse, or improper operation is not covered.

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1.0 INTRODUCTION

1.1 Overview of the Manual

This manual contains operation and maintenance instructions for the *Covaris E and LE-series* instruments (the E220, E220x and LE220). It contains background information essential to the proper use and care of this equipment.

The following definitions apply in this manual:

NOTE:	Inconvenience only if disregarded – no damage or personal injury

CAUTION:	Equipment damage may occur, but not personal injury.

WARNING:	**Personal injury may occur – DO NOT DISREGARD.**

There is also a PDF version of this manual on the system computer (if a computer was supplied with your order). Open the manual by double clicking the manual icon on the desktop.

1.2 Purpose of the Equipment

The E and LE Series instruments are used for high-throughput, sample preparation and extraction. The technology uses controlled and highly focused nonlinear acoustic waves that are delivered to each sample. The systems are designed to work with a variety of closed or open sample vessels including vials, tubes, and multi-well plates. Contact Technical Support for more information. The instruments are not intended for use in diagnostic procedures; for research use only.

CAUTION:	The system is designed to work primarily with closed sample holders. Open vessels may be used; however, the energy should be dramatically reduced as there is potential for leakage and spilling into the water tank. Please contact Covaris Technical Support for guidance in this area if open vessel processing is required.

1.3 Main Components

The main components of the E and LE Series instruments include:

- acoustic transducer
- temperature sensing device
- sample positioning system
- degassing pump
- water bath
- user manual

External components include a notebook computer.

The user is expected to provide a recirculating chiller/heater. Covaris can provide specifications for recommended recirculators.

Figure 1

1.4 Principle of Operation

The Covaris Process generates acoustic shock waves to cause controlled cavitation and acoustic streaming at the focal point within the sample treatment vessel. Acoustic energy has been utilized for many years for a variety of diagnostic, therapeutic and research purposes. Well-known uses of sonic or acoustic energy in materials processing include "sonication", an uncontrolled process of mechanical disruption involving the direct immersion of an unfocused kHz range (e.g., 15,000 cycles per second) ultrasound source into a fluid suspension of the material being treated. There are also specific clinical examples of the utilization of therapeutic ultrasound (e.g. lithotripsy) and of diagnostic ultrasound (e.g., fetal imaging).

Typically, when ultrasound is applied to a bulk biological sample solution (as for the extraction of intracellular constituents from tissue), the treatment causes a complex, heterogeneous, mixture of sub-events that vary during the course of a treatment dose. In other words, the ultrasonic energy may be partitioned between various states. For example, the energy may directly treat a sample; the energy may displace the target and shift the target out of the optimal energy zone; the energy may result in interference, (i.e., a “bubble shield”), that reflects the acoustic energy; or the large particles may move to low energy nodes thereby leaving the smaller particles with more dwell-time in the high energy nodes. In addition, the sample viscosity, temperature, and uniformity will vary during the ultrasonic process, resulting in gradients during the process. The current low frequency, unfocused sonicator-type processes are random and non-uniform. This limits the use of ultrasound in high throughput applications where treatment standardization is required.

In contrast, focused, nonlinear acoustic waves are computer generated and delivered to individually-addressable samples with the E and LE series systems. The mechanical energy imparted on the sample results in a controlled series of compression, rarefaction events. The E and LE-series instruments were designed to provide the researcher with methods and systems to control the delivery of mechanical energy into a biological sample for high-throughput sample preparation applications.

1.5 System Distinctions – E, Ex and LE

All E Series instruments employ an XYZ motion system to move sample plates or racks over the acoustic transducer according to user-programmed methods. E Series instruments, however, are available in several configurations:

- The E220 employs a single focal point transducer to sequentially treat samples as they are positioned over the transducer by the motion system. The E220 may be programmed to a maximum Duty Cycle (acoustic power on/off ratio) of 20.0%.
- The E220x operates identically to the E220, except that Duty Factor may be programmed to a maximum value of 50.0% percent to provide additional treatment power.
- The LE220 operates identically to the E220x, except that a line transducer is employed, producing a line of focused power rather than a single point. Acoustic power is thus applied across the sample plate or rack, treating an entire row of tubes or wells in parallel. The acoustic power is divided approximately across all the samples in a row, so treatment power must be increased accordingly in order to obtain results similar to those obtained with a focused point transducer.

SonoLab operating software automatically detects the type of instrument and adjusts its programming parameters accordingly. The plate or rack graphic seen in the Main and Method Editor screens will also illustrate the point versus line treatment configurations, as illustrated in Figure 2.

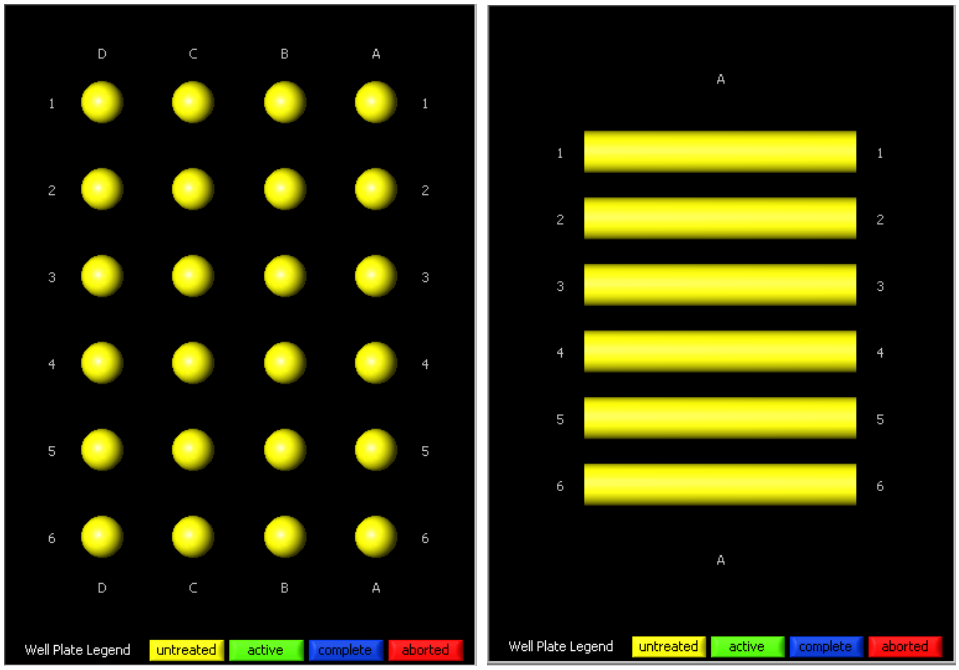


Figure 2 – E and Ex (left) versus LE (right)

Graphics employed in this manual will show the LE well plate configuration, but all instructions are applicable to either E, Ex, or LE systems.

2.0 INSTALLATION

2.1 Unpacking and System Setup

The Covaris system is shipped in one crate, containing the instrument, the instrument supplies, optionally purchased notebook computer, and the selected accessory kit. Remove all wrappings, taking care not to inadvertently discard any parts, accessories, or documents.

Normal precautions in unpacking and reasonable care in handling should be exercised to avoid possible damage to the unit. Visually inspect all external controls, indicators and surfaces to detect any damage that may have occurred during shipment.

The shipping company is responsible for damage to equipment during shipment. If damage has occurred, notify the shipping company immediately to establish proper basis for a claim.

NOTE:	In case of damage do not discard packing material until Covaris has inspected for cause of damage.

WARNING:	**Risk of back injury. The instrument weighs approximately 110 pounds (50 kilograms).**

2.2 Placement of Equipment

The area required for the instrument (see Figure 1) is approximately 23” wide by 30” deep by 19” high (59 cm x 76 cm x 48 cm). Space on or adjacent to the bench is required for a notebook computer. Additional space may be required for a user-supplied recirculator.

Two fans maintain a safe operating temperature in the instrument by circulating air over the internal components. Therefore, place the instrument so that there is a minimum of 1” (3cm) between the rear panel and any wall or obstruction for airflow.

2.3 Power Requirements

The instrument draws a maximum of 500 VA. The Covaris E and LE-Series instrument are configured with a universal power supply. Refer to label on left side of instrument for specific power requirements

In case of a power interruption, the system will halt. The equipment will then need to be restarted. As files or data not saved prior to the power interruption will be lost, the use of an UPS (Uninterrupted Power Supply) is recommended with this system if your site has frequent power surges or loss of power. The UPS should be capable of supplying full 5 amperes at the voltage used.

WARNING:	**To prevent the possibility of electrical shock, always plug the system into a grounded power source.**

2.4 Set-up Procedures

2.4.1 Connections

Ensure that the user-supplied chiller is connected to the CHILLER IN and OUT ports on the left side of the instrument using the Covaris supplied hoses. The hoses are setup with quick disconnect fitting to be attached to the instrument and hose clamps for the user supplied chiller

Ensure there is a USB cable connected from a USB port on the computer to the USB port on the instrument (located above the power switch on the right side, near the back of the instrument).

2.4.2 Powering Up the System

Power up the Covaris system by moving the power switch to the “1” position. If the computer is supplied by Covaris, turn it on. Open the SonoLab software by double clicking the SonoLab icon on the desktop.

NOTE:	To ensure connection between the laptop and instrument, please wait 30 seconds after powering the system on before starting SonoLab 6.2. Re-home the system daily by closing and restarting SonoLab. This will correct any accumulated misalignments in the system.

2.4.3 Control Buttons

There are two control buttons located on the right side of the instrument as shown in Figure 3. The red STOP button can be depressed and held for 1 second at any time to halt operation. The green DOOR button, when lit, can be depressed to allow the operator to open the safety enclosure door (See section 3.4).

Figure 3

2.4.4 Water Bath Configuration

The water bath tank needs to be oriented properly when inserted into the system. The bath should be oriented with the label “Insert this end” at the back of the tank (see Figure 4). The "waterline" label will be displayed on the front side of the tank.

Figure 4

CAUTION:	The magnetic safety interlock will not allow the instrument to operate if the water tank is not oriented properly and fully inserted.

Fill the water bath to the water line on the side of the tank, as the water level will rise when the transducer is submerged. Only distilled or deionized water should be used to fill the water bath.

CAUTION:	Never run a method without a water bath. This will permanently damage the transducer.

CAUTION:	Using anything besides distilled or deionized water in the water bath is a misapplication of the equipment and will void the warranty.

The system includes a water level sensor. If the water level is too low, a warning massage will be issued by the software. In addition, the degas pump will not operate for more than 10 seconds if a low water level is detected. This is to protect the pump from running without water.

Prior to running a method, the water bath should be degassed for 45 minutes. Degas the system by lowering the transducer (click the START HOMING button in the Initiation Sequence or click the LOAD PLATE button on the MAIN PANEL of SonoLab). Then click the DEGAS ON button.

NOTE:	The DEGAS ON button will not engage the degassing pump if the transducer is in the SERVICE BATH position

NOTE:	The degas pump will turn off after 10 seconds if the water tank is not filled with water to the fill line.

3.0 SYSTEM DESCRIPTION

3.1 General Overview

The *Covaris E and LE220 systems*, as shown in Figure 1, are custom instruments for high-throughput sample preparation and compound management. The system is comprised of the Treatment System which delivers the energy to the sample, the Safety System which provides intelligent interruption during the “treatment”, and the Computer and the Application Software which provide the user interface to the entire system.

This instrument is a very powerful, high-energy system. It is designed to disrupt and homogenize biological tissue, extract biochemicals, and accelerate reagent dissolution.

3.2 Treatment System

3.2.1 Acoustic Transducer

The acoustic transducer delivers a high intensity, focused, computer-controlled acoustic shock wave to a sample. This is called the “treatment”. The treatment of all samples in a tube rack or well plate is called a method.

WARNING:	**Objects at the focal point of the transducer will be exposed to high energy when the system is activated.**

3.2.2 Temperature Sensors and Temperature Control

The water bath sensor, a 5cm metal probe, monitors the temperature of the water bath. The water bath temperature limits can be set to values between 0º C and 45º C. The water bath temperature is controlled by connecting an external recirculating chiller/heater to the ports in the back of the system. The recirculator will pump a temperature-controlled fluid through a stainless steel loop immersed in the water bath.

NOTE:	Although the instrument reports the bath temperature via the Temperature Sensor, it does not control the recirculating chiller. Typically, for most applications (e.g., gene expression, proteomics, compound dissolution, and drug metabolism studies) the temperature of the recirculating chiller is set to 15 degree C with the temperature of the water bath equilibrating at 18 degree C.

CAUTION: A recirculating chiller is required for efficient and controlled operation of the E and LE-series instruments.

3.2.3 Sample Positioning System

The major components of the system include:

The sample positioning system (x, y, z axes)

The transducer positioning system (z' axis)

The sample holder plate

The robotic interface

Figure 5 shows the sample holder plate with the A1 position identified.

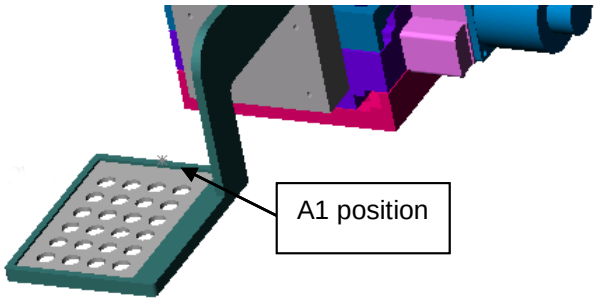


Figure 5

3.2.4 Water Bath and Degassing System

The water bath holds approximately 1.3 gallons (4.92 liters) of distilled or deionized water. The water level is indicated by a label on the front wall of the water bath. The left side of the label ("FILL") indicates the water level when filling the bath. The right side of the label ("RUN") indicates the water level when the transducer is submerged.

The water level will be dictated by the tube rack. If Covaris has provided an Instruction for Use (IFU) for your tube rack, please refer to it for the recommended water level. If no water level is specified, fill the water bath to the "0" level.

WARNING: The water bath is heavy when filled. Use caution when moving it.

The degassed, temperature controlled water provides an efficient energy couplant from the transducer to the sample to be processed. The water bath must be degassed for approximately 45 minutes to ensure proper operation of the instrument. The degassing pump will start automatically as the transducer is submerged. Its ON status is indicated by a green indicator on the SonoLab main screen. If the degas pump has been turned OFF, it can be restarted by clicking the DEGAS ON button.

The degassing system consists of a dip tube with six small holes, a positive displacement pump, and an outlet nozzle. Dissolved gasses and vapors are reduced in the water by pulling the water through the small holes in the dip tube and pumping the resulting coalesced bubbles out the outlet nozzle before they can re-dissolve. During normal operation, there should be an intermittent stream of bubbles coming from the outlet nozzle. If no bubbles are visible for several minutes, the six small holes in the dip tube may be plugged. If this occurs, the dip tube may be removed and the holes cleared with a fine wire (e.g., 26 Gauge needle).

3.3 Computer

The computer uses the Microsoft™ Windows® XP Operating System or Windows 7 with SonoLab™ application software.

3.4 Safety System

The safety system protects the user from hazards associated with the acoustic treatment system and the moving parts in the sample positioning system.

The safety system prevents operation of the instrument if the front door is open, the water tank is not in place, or the robotic interface is not enclosed. The front door can only be opened when the green light on the DOOR button is lit. Once lit, pressing the button will allow the door to be opened. There is also a red STOP button on the right of the safety enclosure (see Figure 3). Depressing the STOP button for one second (or clicking on the STOP button in SonoLab) halts all operations. The Process Stopped Dialog Box appears, which allows the user to either Resume or Abort the current operation as shown in Figure 6. Note that Resume is viable only when the SonoLab STOP button has been used. Methods halted with the safety enclosure STOP button cannot be restarted.

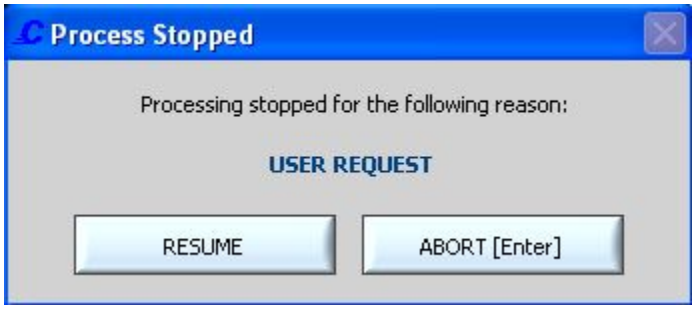


Figure 6

NOTE**:** The door may be opened in the event of a power failure by inserting a special tool provided with the instrument into the hole in the enclosure above the safety interlock and rotating clockwise 90 degrees. The instrument will not operate if the safety interlock is disabled. The tool is stored in a clip on the back of the instrument.

3.5 Water Bath Temperature

The software monitors water bath temperature. If the bath temperature is beyond user defined limits, while a method is running, the method is halted and the following dialog box will appear:

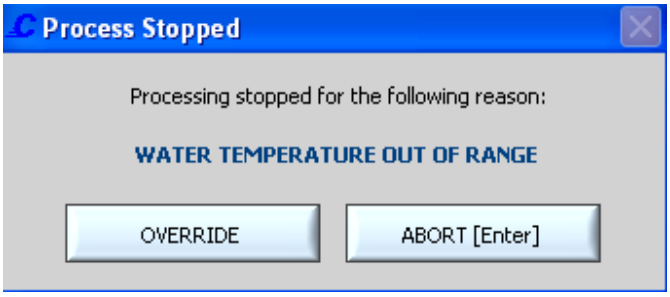
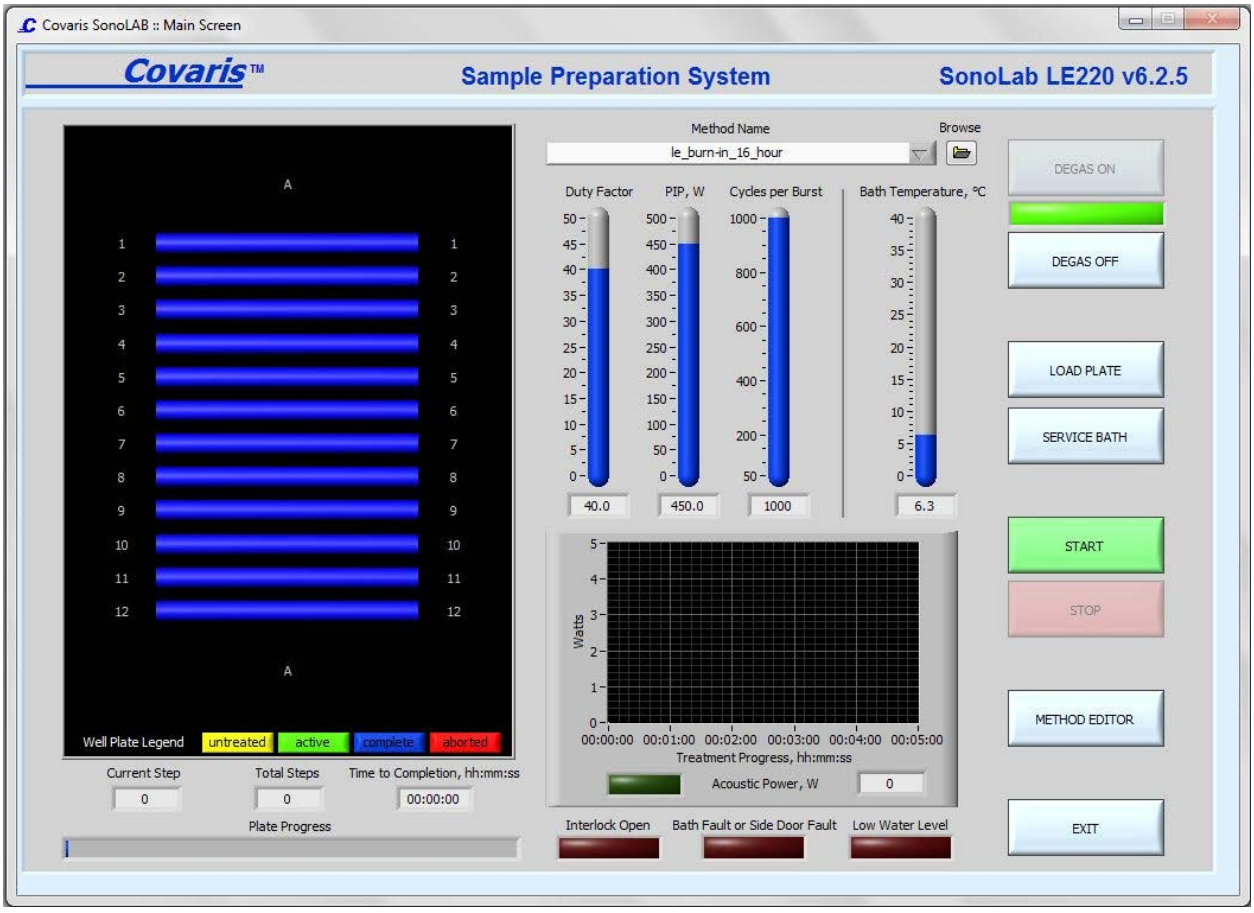


Figure 7

3.6 Application Software

3.6.1 Main Panel



Software Panel 1: MAIN PANEL (LE220 application shown)

Motion Buttons

The motion buttons move the sample holder and the transducer. They operate as follows:

DEGAS ON starts the degas pump

DEGAS OFF turns the degas pump off

LOAD PLATE lowers the transducer from a raised position (SERVICE BATH position) and moves the sample holder to the manual load position.

SERVICE BATH raises the transducer and sample holder out of the water bath.

METHOD EDITOR button will take the operator to the *Method Editor Panel* which allows the user to create and/or modify a method.

START button starts running a method. After selecting the button, a warning dialog box opens to ensure the system is ready to proceed. The warning reminds the user to check that there is water in the water bath, that the system has been degassed, and that there are samples in the sample holder. See the warning screen below:

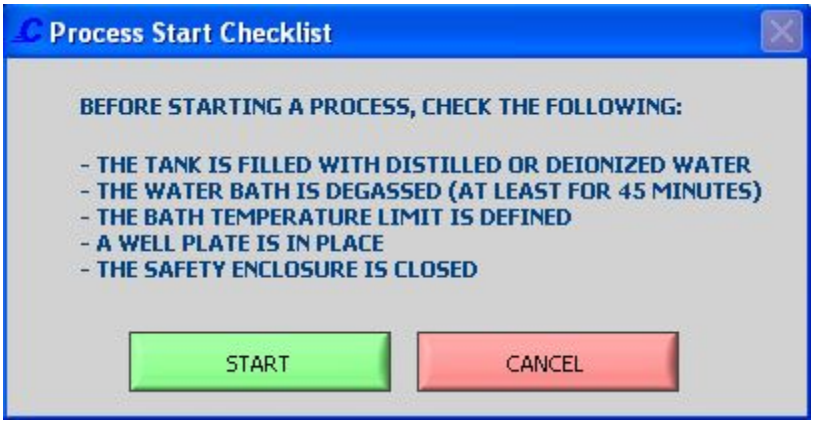

Process Start Checklist
BEFORE STARTING A PROCESS, CHECK THE FOLLOWING:
- THE TANK IS FILLED WITH DISTILLED OR DEIONIZED WATER
- THE WATER BATH IS DEGASSED (AT LEAST FOR 45 MINUTES)
- THE BATH TEMPERATURE LIMIT IS DEFINED
- A WELL PLATE IS IN PLACE
- THE SAFETY ENCLOSURE IS CLOSED
START
CANCEL

Figure 8

If the user is ready to proceed, click the START button.

STOP halts the operation of the sample positioning system. (Methods may then be aborted or resumed.)

NOTE: The STOP button on the instrument and the STOP button on the display perform the same function, however methods halted with the instrument STOP button cannot be resumed.

The EXIT button will exit the program.

The Status Indicator Lights

ACOUSTIC POWER indicates that the transducer is active.

LOW WATER LEVEL indicates that the water sensor and transducer are not immersed.

INTERLOCK OPEN indicates the software senses that the mechanical safety interlock is in the open position.

BATH or SIDE DOOR FAULT indicates that the water tank or the side door (robotic systems only) is not in place or properly secured.

DEGAS indicates the degassing pump is on. (Located below the DEGAS ON button.)

While a method is running, the following indicators display current treatment parameters:

BATH TEMPERATURE displays the temperature of the water bath. The thermometer graphic, normally blue, will turn red if bath temperature exceeds the temperature limits set by the user in the method.

DUTY FACTOR, PIP (PEAK INCIDENT POWER), and CYCLES PER BURST show the parameters for the current well being processed. (Refer to section 3.6.2)

ACOUSTIC POWER GRAPH shows the acoustic power being delivered to the sample for each treatment.

Plate Map Graphic

Each row of wells is represented in the plate map. Untreated wells are yellow. A well undergoing treatment is green and once completed the color changes to blue. If a treatment is aborted, the row will appear red.

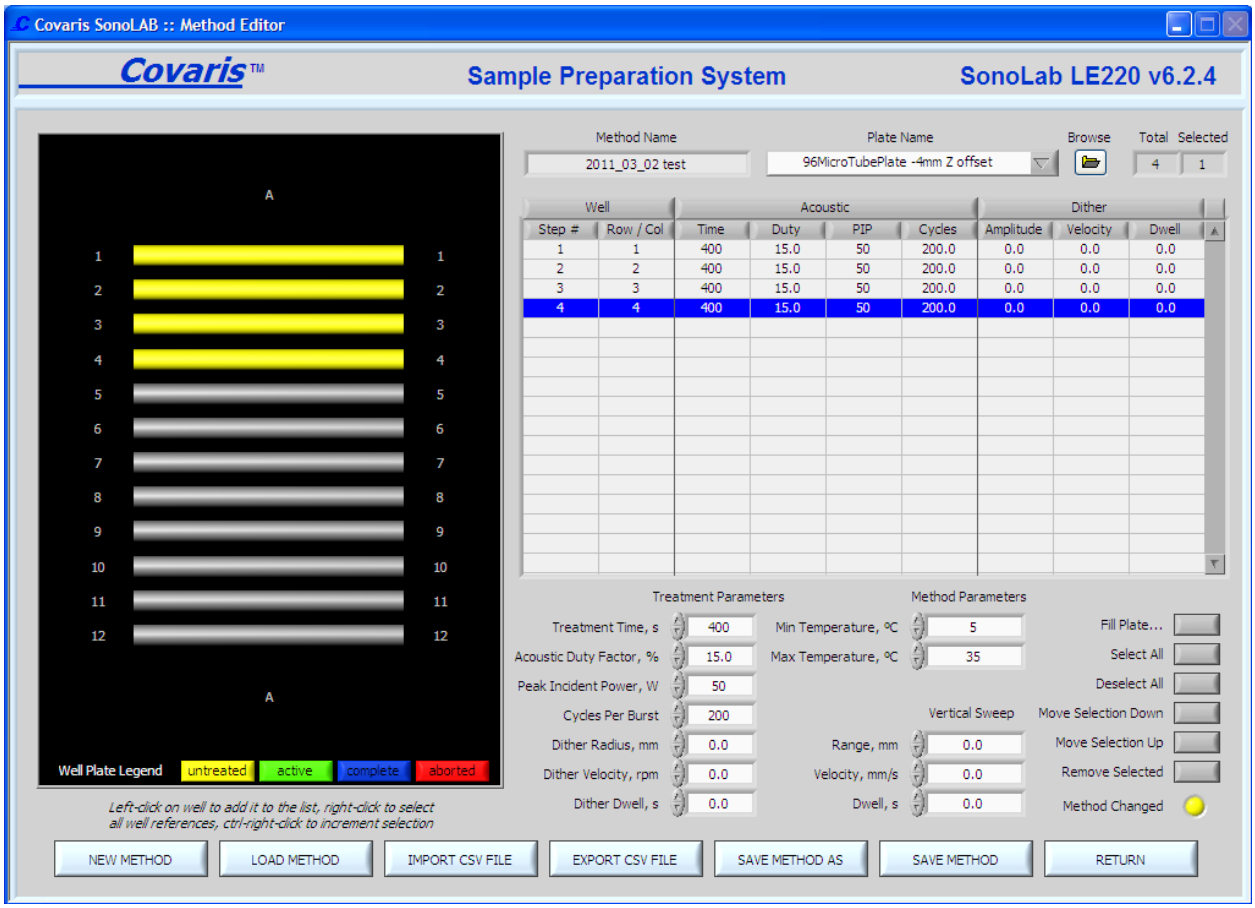
Acoustic Treatment Graph

The DUTY FACTOR, INTENSITY, and CYCLES/BURST indicators display the treatment parameters being applied to each well during a method run. These parameters are programmed into each method by use of the Method Editor Panel (see section 3.6.2).

Progress Bar

Indicates the percent completed of the currently running method (as a percent of the total calculated time).

3.6.2 *Method Editor* Panel



Software Panel 2: METHOD EDITOR PANEL

This screen allows the user to select either a previously created method (and its associated parameters) or to create a new method. On the left side of the screen are the controls to set-up the well plate.

Left clicking on a table entry highlights that entry. The shift and control keys may be employed to highlight multiple entries. The Treatment Parameters may be edited for all highlighted table entries.

SELECT ALL and DESELECT ALL manipulate highlighting of steps in the Method Table. The MOVE Buttons move highlighted entries within the table.

The FILL PLATE button creates multiple sequences per well by applying the treatments highlighted in one or more rows in the table using the ordering of the selected sequence.

SELECT ALL will highlight all entries in the list of treatments. Edits made to acoustic parameters will apply to all highlighted entries.

DESELECT ALL will remove highlighting from all entries in the list of treatments.

MOVE SELECTION DOWN will move the highlighted entries down one position in the list.

MOVE SELECTION UP will move the highlighted entries up one position in the list.

REMOVE SELECTED will delete highlighted entries from the list.

Note that edits to method parameters will cause the METHOD CHANGED indicator to illuminate until these edits are either undone or saved.

Treatment Parameters

For a new or an existing method, the user may define and edit the individual row parameters or sequence in the control panel.

ROW/COL is the current treatment position in the well plate. The set of parameters defined for the well is a treatment.

TIME is the amount of time (sec) to apply the parameters or to treat a sample.

Acoustic energy is produced in bursts and the system allows the user to modify this energy to maximize the effects on the treatment of samples for any particular application. Contact Covaris Technical Support for recommendations regarding these settings.

DUTY FACTOR is defined as a percentage. This is the percentage of time that the transducer is “on” and creating acoustic waves. Average acoustic power will increase with greater Duty Cycle. Minimum is 0.1%. Maximum is 20.0% for E and 50.0% Ex and LE.

PIP (PEAK INCIDENT POWER): The instantaneous power in Watts transmitted to the acoustic transducer during the “on” time of each acoustic burst. The larger the number, the more energy each acoustic wave has. Minimum is 3.0 and maximum is 500.0 Watts.

CYCLES PER BURST: The number of waves generated by the transducer in a burst. For example, 100cpb at 10% duty factor is a wavetrain of 100 “on” energy cycles followed by 900 “off” energy cycles. Minimum is 50 and Maximum is 1,000.

Dithering is the controlled horizontal circular movement of the treatment vessel around a center point during processing to mix the sample. For horizontal dithering, the following parameters can be set:

RADIUS is the radius (in mm) the vessel moves in a circular motion.

VELOCITY is the speed (in rpm) of this movement of the vessel.

DWELL is the time the vessel remains at the center point between dithering operations.

CAUTION:	Method progress will halt if these values exceed the maxima allowed by each well plate definition. Test dithering functions before using them in on valuable samples. Contact Covaris for guidance if needed.

Method Parameters

FREQUENCY SWEEPING: The acoustic frequency sweeps through a range during processing. This is the default setting for the E and LE-series instruments. As plastic readily absorbs acoustic energy, this lower power setting is more appropriate for method development with plastic vessels. Frequency sweeping is both preferred and recommended for plastic sample vessels.

VERTICAL SWEEP moves the sample up/down during processing.
VERTICAL +/- is the vertical movement in mm.
VERTICAL RATE is the frequency in cpm (cycles per minute).

MAX and MIN TEMPERATURE allow the user to select water bath temperature limits for the treatment. The temperature limits can be set to a value in the range of 0º C and 45º C. If the water temperature is beyond the specified limit, the BATH thermometer on the main panel will turn red and processing will be halted. It can be restarted by selecting OVERRIDE in the temperature warning dialog box.

General Operation Buttons

The following buttons are employed for creating, importing, exporting and saving methods. Note that edits to method parameters will cause the Method Changed indicator to illuminate until these edits are either undone or saved.

NEW METHOD opens a new method with no treatments yet defined for any wells.
LOAD METHOD opens an existing method (*.e1proc file) and displays it on the Method Editor panel.
IMPORT CSV FILE and EXPORT CSV FILE features have not been implemented.
SAVE METHOD AS saves the currently displayed method parameters to a new or existing method (*.pro) file.
SAVE METHOD saves the currently displayed method parameters to the method (*.e1proc) file name displayed in Method Name.
RETURN closes the Main Panel. Note that you may be prompted to save or discard any unsaved edits before returning to the Main Panel.

4.0 SYSTEM OPERATION

4.1 Start-Up Procedures

Chiller Connection

The recirculating chiller (required) should be connected to the treatment system using the hoses supplied. The chiller inlet is connected to the fitting labeled “IN” and the chiller outlet is connected to the fitting labeled “OUT” located on the left side of the instrument.

Water Bath

Special care needs to be taken when changing the water bath. First, select the SERVICE BATH button to raise the transducer. Then carefully pull out the acrylic water tank from the instrument base plate and empty it. Fill the water tank and place it back into its position, ensuring that it is oriented properly in the instrument and the water is at the correct level (see section 2.4.3).

WARNING:	The water bath is heavy when filled. Use caution when moving it.

CAUTION:	Never run a method without a water bath; the system should detect this and refuse to operate, but a lack of water risks permanent damage to the transducer.

Degassing

The *Covaris* system requires that the water bath be degassed to maintain repeatable acoustic energy coupling, therefore, the pump should be running whenever samples are being treated. This is especially critical for high intensity applications such as solid tissue homogenization. At room temperature, the water bath should be degassed for at least 1/2 hour prior to running a treatment method. Select the DEGAS PUMP button on the Main Panel. Ensure the water bath is filled to the water level before turning on the pump; otherwise, damage to the pump may occur.

NOTE:	The degassing pump will turn off after 10 seconds if the water tank is not filled with water.

Manual Loading of the Multi – Well Plate Holder

Select the LOAD PLATE button on the Main Panel to raise the multi-well plate holder. The system will position the stage above the water bath.

Fill the tube rack with the sample vessels to be run. If required, place the hold-down plate over the vessels. The hold-down plate ensures that the vessels stay in place for correct processing.

The plate should be loaded on the multi-well plate holder with the A-1 position in the upper right corner. See Figure 5.

If not all the wells are filled with samples to be treated and the method is high intensity, load the unused positions with empty vessels to reduce splashing.

NOTE**:**	Ensure the well-plate is seated flush onto the well-plate holder to insure the samples are correctly aligned with the focal point of the transducer.

4.2 Creating and Modifying a Method in the Method Editor Panel

To open an existing method to run or to modify, select METHOD EDITOR, click on LOAD METHOD and choose from the list of previously saved set-ups.

To create a new method, select the METHOD button.

Select the Well Plate Configuration from the PLATE NAME menu.

Parameters may now be selected in the Treatment Parameters area.

Select the Current Well Parameters

Click on any well; a treatment can be configured using the Treatment Parameters.

Treatment parameters for additional wells may be programmed by clicking on those wells and using the Treatment Parameters controls. Well treatments may be copied across an entire plate by employing the FILL PLATE button. This feature also allows several choices for the order in which wells are listed in the method. Wells will always be treated in order as displayed in the treatment entry list.

Save the method by selecting the SAVE METHOD button. The system will prompt the user to name the METHOD.

4.3 Running a Method Manually in the Main Panel

Load the samples first by selecting the LOAD PLATE button. The motion system will move the sample holder tray to the front of the enclosure and the green DOOR button will illuminate. Press the DOOR button to open the door and position the samples on the tray.

Click the pull-down menu button under the Method Name. Select from the list of defined methods.

Click the START button. The system will run the treatment displayed in the Method Name box.

CAUTION:	If the computer loses connection with the instrument during processing, the method will stop.

The following Main Panel indicators provide system status information:

The green indicator between the DEGAS buttons will illuminate when the degas pump is on. This pump should be run for at least 45 minutes prior to running a method.

On the WELL PLATE MAP, each well will appear yellow prior to treatment, green during treatment and blue following treatment.

The CURRENT STEP, TOTAL STEPS and ESTIMATED TIME TO COMPLETION displays monitor progress through the method.

The ACOUSTIC POWER indicator and graph indicate when acoustic treatment is being applied, and its approximate power.

The DUTY FACTOR, PIP, AND CYCLES PER BURST graphics show the treatment parameters being applied to a well when the Acoustic Power indicator is illuminated.

INTERLOCK OPEN will illuminate when human access is occurring in the acoustic treatment area. Acoustic power and internal motion will be suppressed whenever an INTERLOCK OPEN condition is detected. Specifically, this will occur either when the front door is open, and/or when the side robot interface door is open with no robot interface in place.

4.4 Manual Overrides

There is a STOP Button on the main SonoLab screen. This will halt the current method. The method may also be stopped by pressing and holding the red STOP button on the instrument for one second. The green DOOR button will light once the method has stopped.

To restart, the system will prompt the user with a message to either RESUME or ABORT the method. If the door is not closed, selecting RESUME will prompt the user again to RESUME or ABORT. Selecting RESUME with the safety system closed will continue the method where it was interrupted. Selecting ABORT terminates the method.

NOTE**:**	Methods stopped with the safety enclosure STOP button cannot resume via the RESUME function in SonoLab. To pause and resume a method, use the SonoLab STOP button.

4.5 Shut-Down Procedures

Raise the transducer out of the water bath by selecting the SERVICE BATH button. Sponge out the “bowl” of the transducer to remove water. Empty the water bath. If the instrument will be idle for an extended time, lower the transducer and run the degassing pump with an empty water bath for 10 seconds to drain the degassing lines.

CAUTION:	Leaving water in the water bath or degassing lines can cause algae growth in the tank and lines which may interfere with sample processing.

Exit the SonoLab software by selecting EXIT on the main panel.

Select Shutdown from the START menu on the Windows screens and select the option “Shut down the computer”. The computer will power down. The instrument can be powered down by moving the power switch to the “0” position. The power switch is located at the rear of the instrument on the right side.

CAUTION:	Failure to raise the transducer from the water bath when not in use may result in permanent damage to the system.

5.0 SYSTEM SPECIFICATIONS

Model	E220, E220x, LE220
Treatment System	Bench-top: high intensity acoustic transducer, sample positioning system, temperature monitoring device, circulation pump, and water bath with safety enclosure. Dimensions: 23” W x 30” D x 19” H (59cm x 76cm x 48cm) Weight: approximately 110 lbs (50 Kg)
Computer	Notebook computer (may be supplied by Covaris or the user)
Operating System	Microsoft Windows XP Professional or Windows 7 (32-Bit Recommended)
Application Software	Covaris SonoLab 6.2
Data Input	Keyboard, mouse
Chiller	Chiller/heater re-circulating system - not included. Connect with the 3/8 inch I.D. hoses and quick connect fittings supplied
Power Requirements	100-240 V~ 50-60Hz 500VA maximum
Water Temperature	+4.0°C to +50.0°C

6.0 MAINTENANCE

6.1 Water Bath

6.1.1 Recommendations for Daily Maintenance

Only distilled or deionized water should be used to fill the water bath. The water bath should be emptied every day to avoid algae growth. Do not use an algaecide.

The water level is dependent upon the sample vessel type. There is a water line in the tank to help determine the depth required. Refer to the technical notes appropriate to the sample vessel to determine water depth.

6.1.2 Recommendations for Monthly Maintenance

Periodically, the water bath and degassing lines may be rinsed with a 10% solution of Clorox bleach. The resultant sodium hypochlorite solution is ~ 0.5% NaOCl. With this solution in the water bath, lower the transducer and run the degassing pump for a few minutes. Repeat with fresh water in the water bath.

6.2 Transducer

When not in use, the transducer should be raised out of the water bath and the transducer should be sponged out with a soft lint-free cloth or sponge to remove any residual water. Keeping the transducer dry between applications will help prevent corrosion and is strongly encouraged to ensure optimal performance.

Use extreme care when handling the transducer. Permanent damage could result if anything is dropped onto the face of the transducer or if the face of the transducer strikes something.

Permanent damage could also result if the transducer is overheated, (i.e. running the transducer without a water bath).

WARNING: Do not attempt to remove the transducer as irreparable damage may occur.

6.3 Safety System

Test the system periodically. Make sure that the operation is halted when the STOP button is depressed.

WARNING: Any failure of the Safety System must be reported immediately to Covaris. DO NOT attempt operation if the Safety System is malfunctioning.

6.4 Degassing the System

The degassing pump will shut down after 10 seconds without a water bath because running the pump without water will damage the pump.

Check that there are bubbles coming from the end of the degassing tube. Remove the dip tube (inlet tube) if necessary and examine it under a microscope to clear any clogged holes.

For long-term storage, all water should be removed from the degassing pump lines. This can be done by running the degassing pump for 5 to 10 seconds with an empty water bath.

6.5 Relocation of the System

Should it be necessary to move the instrument, for whatever reason, the system needs to be properly prepared. This procedure describes the steps necessary to relocate Covaris LE-Series instrument. If the instrument is to be moved off site, a crate may be obtained from Covaris.

6.5.1 Prepare the System

- Raise the transducer ("Service Bath" position) and sponge out the bowl of the transducer. Empty the water tank and reinstall it in the instrument
- Lower the transducer and run the degas pump. Run the pump for 10 seconds, enough to drain the water from the pump lines.
- Raise the transducer ("Service Bath" position) and empty the residual water from the tank
- Leave the transducer in the up position ("Service Bath" position).
- Save the current method and close SonoLab software by selecting EXIT on the main panel.
- Shut down the computer from the START menu.
- Power down the instrument by moving the power switch to the "0" position. The power switch is located at the rear of the instrument on the right side.
- Disconnect the instrument power cable, the USB cable, the mouse and the computer power supply. Gather these items and keep with the instrument.
- Disconnect the Chiller from the instrument. Drain the water in the chiller into a bucket through the drain fitting. The hoses can be drained by depressing the valve in the center of each quick-connect fitting and holding over a bucket.
- Protect the chiller quick-connect fittings on the lower left side of the instrument from damage by taping foam or bubble wrap over them.

6.5.2 Move of the System

- If the instrument is to be moved off-site, procure a crate from Covaris. Pack the instrument in the crate using the provided foam inserts.
- Protect the system and computer with a light cloth or plastic to prevent dust contamination.
- Be sure all the components are accounted for, including tube holders and user manual.
- Transport the instrument and accessories. At least two capable people are required to lift and place the instrument. The instrument weighs approximately 50kg (110 lb). The chiller is also heavy.

6.5.3 Reinstall the System

- Instructions for reinstalling the instrument are contained in Section 2 of the User Manual. If the manual is missing, obtain a new copy from Covaris.

7.0 TROUBLESHOOTING

System Trouble Analysis Chart

SYMPTOM	PROBABLE CAUSE	POSSIBLE SOLUTION
Error message “Unknown Instrument Type”	Device Com ports were assigned incorrectly	Go to C:\Covaris\E-Series v6.2\Program to open the SonoLab configuration file, “SonoLab E-Series.ini”. Replace: ComPortMotion=usb to ComPortMotion=COM4 & ComPortSound=usb to ComPortSound=COM3 See **Figure 8** of Appendix B in this manual for COM Port assignment verification.
Degassing system not generating bubbles.	1. Degassing dip tube may be clogged 2. Pump not working	1. Remove and backflush under running water 2. Examine the six holes (e.g., using a stereo microscope) to see if clogged and use a pin to unclog them 3. If pump does not run, contact Covaris.
Incorrect positioning, inconsistent treatments	Home position lost, system misalignment	Home system by selecting “initialize system” from the pull-down menu on the main panel, or by restarting SonoLab.
Safety interrupt	1. Water tank is misaligned 2. Door is open. 3. STOP button is depressed 4. Safety interlock is bypassed	1. Check tank alignment 2. Check door 3. Check safety interlock
Sample vessel hits the water jet nozzle	1. System has lost the home position. 2. Incorrect nozzle position	1. Home the system 2. Reposition water jet manually
Error message that system not shut down correctly	SHUT Down command in Windows XP not used before rebooting	1. Follow instructions on screen 2. Use SHUT DOWN command
Delay in powering degassing pump on/off	If a method is running, there is a delay in powering the degassing pump on/off	Wait until method completes before powering the degassing pump on/off
Values for Dithering and Vertical Sweep are different from the values that were entered	Values entered are larger than the limits for a particular Well Plate	Use lower values

APPENDIX A: SonoLab 7 And 6.2 Software Installation on a Windows XP Or 7 Computer

> **NOTE:** SonoLab version 6.2.4 and later requires SonoLab 7 to be installed first and be resident in the computer prior to installing 6.2.x. SonoLab 7 need only be resident in the computer - it does **not** need to be running. If you require a copy of SonoLab, please contact Covaris.

Install SonoLab 7 on the Windows XP or 7 computer:

1. Begin with the USB cable disconnected and the instrument powered off.
2. Copy or extract the SonoLab 7 installer onto the computer desktop.
3. **For Windows XP:** Double left click setup.exe in the SonoLab 7 folder to launch the software setup process.

   For Windows 7: *Right* click on setup.exe in the SonoLab 7 folder and choose “Run as Administrator”.

   Follow the installation wizard step by step until the installation is finished.
4. Install Drivers as specified in the next APPENDIX. When the driver installation is complete, proceed to the next steps.
5. **For Windows XP:** Double click on the desktop SonoLab 7 shortcut to start SonoLab 7™.

   For Windows 7: First start only - Locate the SonoLab,exe executable file in the location C:\Program Files\Covaris\SonoLab, right click on SonoLab.exe and choose “Run As Administrator”. Once the application is running, quit SonoLab. SonoLab may then be started normally from the desktop icon.
6. When running SonoLab 7, follow any firmware update instructions that appear. Quit SonoLab 7.

Install Covaris SonoLab 6.2.x E and LE-series software on the Windows XP or 7 computer:

7. Begin with the USB cable disconnected and the instrument powered off.
8. Copy or extract the SonoLab 6.2 installer onto the computer desktop. Locate setup.exe in the Volume folder and double-left click. The installer will launch in about 30 seconds.
9. Simply click Next to proceed through the installation process. When queried, accept any software license agreement and click Next to continue with the installation.
10. When installation is complete, click Finish. Restart the computer if requested by the installer software. A shortcut to SonoLab 6.x should appear on the computer desktop
11. When the computer restart is complete, turn the instrument power ON and plug the USB cable into a USB port on the computer.
12. Wait 30 seconds, then double click on the shortcut to start SonoLab 6.2 software.

13. To install the standard set of plate definitions, open the installer folder and navigate to the Plates folder. Open this folder. In this folder are located standard plate definitions that can be employed to create treatment processes in the SonoLab Method Editor.

14. Highlight the plate definitions and copy the files to the following location on the computer:

    C:\Covaris\SonoLab v6.2\Plates

15. To install an example of a treatment process, open the installer folder and navigate to the Processes folder. Open this folder. In this folder are located treatment processes. Copy the files to the following location on the computer:

    C:\Covaris\SonoLAab v6.2\Processes

    This method may be loaded from the Method Editor screen in SonoLab and run as an example.

NOTE: Laptop standby and hibernate modes should be avoided while operating the SonoLab software. To ensure this, from the start menu, open Control Panel, then Power Options. Select a Power Scheme that sets System standby and System hibernate times to Never. If standby and hibernate modes remain enabled, then the SonoLab software should be closed and the system powered down whenever the system is not in use.

NOTE: SonoLab software was has not been validated running concurrent with other application software. We therefore recommend that no additional application software is loaded. Wireless Network Connections on the Laptop should be disabled to avoid loading of any unwanted software. Go to Network Connections in the Control Panel. Highlight the Wireless Network Connection and select “disable this network device”.

APPENDIX B: Covaris 220 Series Instrument Driver Update Procedure for Windows 7

Windows Drivers are software files required to support communications between a computer and any USB (Universal Serial Bus) device. Specific drivers are needed to support communications with the E/LE220.

A- If a laptop is configured for internet access (either via wireless link or Ethernet cable) and automatic driver updates, then the first time the E/LE220 is connected to a laptop and powered ON, Windows will automatically install the necessary driver and provide updates as these drivers are installed.

- Please note that drivers for several devices within the E/LE220 will be installed sequentially. These include a Generic USB Hub, Covaris RF Controller Board, Covaris RS-485 Port, Covaris Serial Port 2 and Covaris Serial Port 3.
- Please allow all of these drivers to install and for Windows to confirm successful installation of all drivers before starting SonoLab 7.

B- If the laptop has internet access, but is not configured for automatic updates, then either change this setting to automatic, or instruct Windows to search for each driver automatically as this query appears.

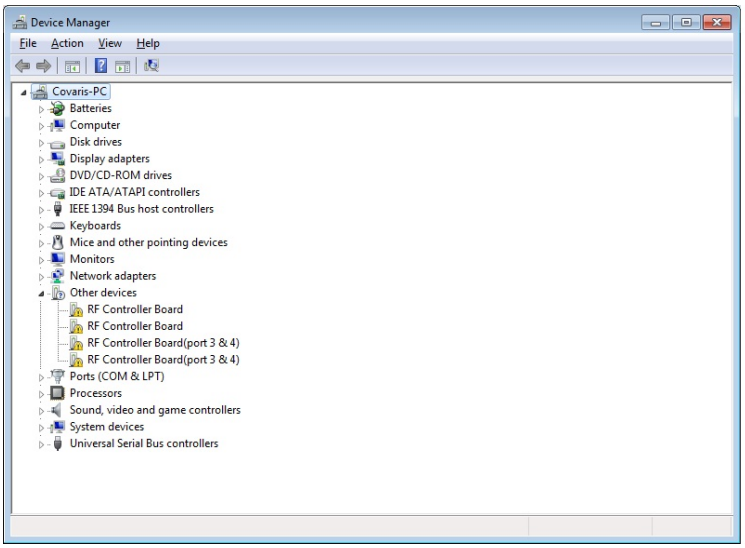
C- If the laptop does not have internet access, please follow these steps:

a. Connect a 220 Series instrument to the instrument control computer using a USB Cable.

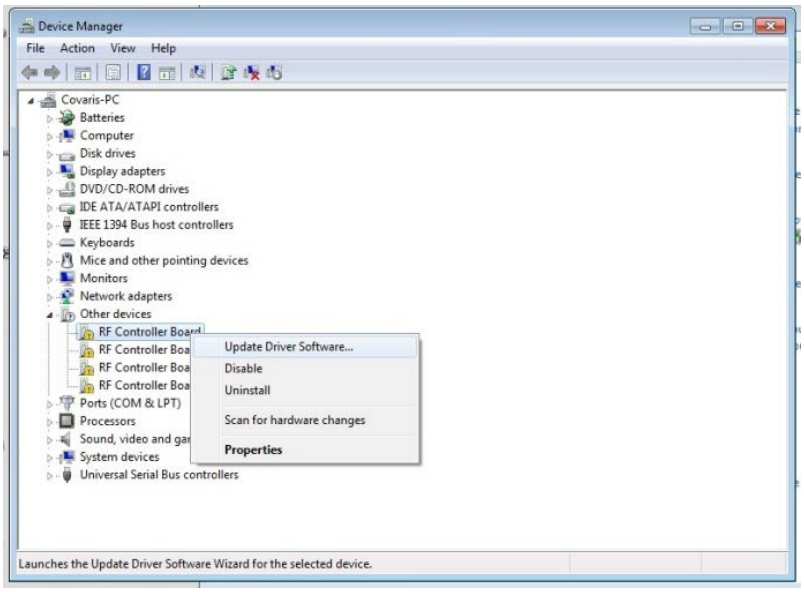
b. Plug the instrument in and power it up.

c. Click on the **Start menu\ Control Panel\Hardware and Sound.**

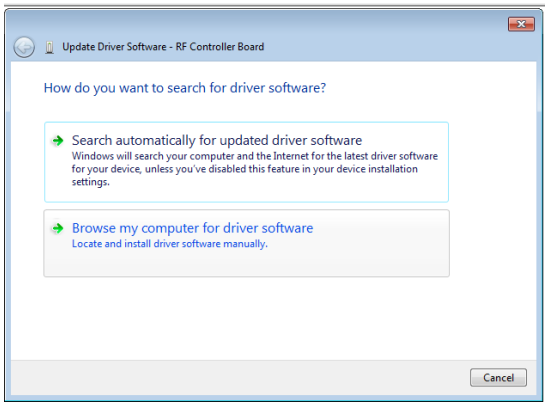
d. Under **Devices and Printer**, click on **Device Manager**. The Device manager window will appear.



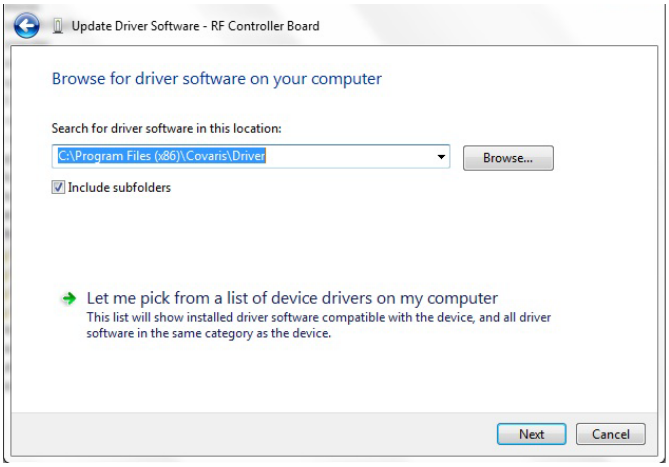
e. Under **Other devices**, right click first device RF Controller Board and select **Update Driver Software.**



f. When the driver software update dialog opens, select the option to 'Browse my computer for driver software'.


Update Driver Software - RF Controller Board
How do you want to search for driver software?
Search automatically for updated driver software
Windows will search your computer and the Internet for the latest driver software for your device, unless you've disabled this feature in your device installation settings.
Browse my computer for driver software
Locate and install driver software manually.
Cancel

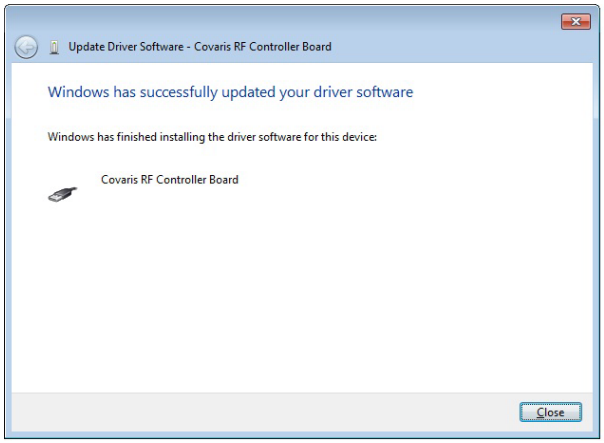
g. Point the file open dialog to "c:\Program Files\Covaris\Driver" to find the necessary instrument drivers.


Update Driver Software - RF Controller Board
Browse for driver software on your computer
Search for driver software in this location:
C:\Program Files (x86)\Covaris\Driver
Browse...
Include subfolders
Let me pick from a list of device drivers on my computer
This list will show installed driver software compatible with the device, and all driver software in the same category as the device.
Next
Cancel

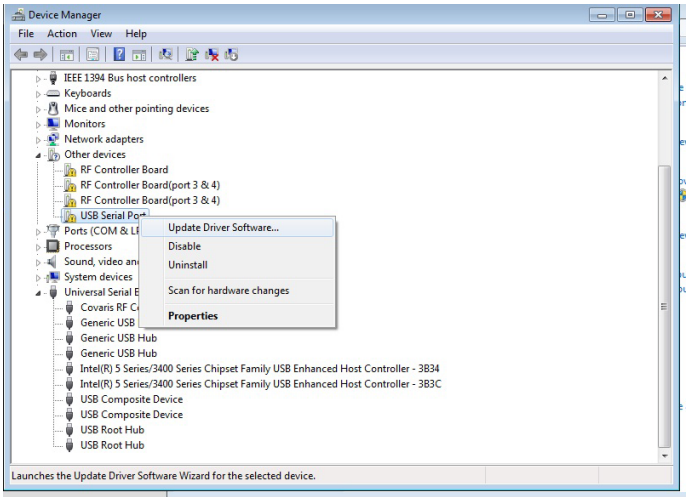
h. Click next. You will be notified that Windows can't verify the publisher of this driver software. Click on "Install the driver software anyway".

Windows Security
Windows can't verify the publisher of this driver software
Don't install this driver software
You should check your manufacturer's website for updated driver software for your device.
Install this driver software anyway
Only install driver software obtained from your manufacturer's website or disc. Unsigned software from other sources may harm your computer or steal information.
See details

i. Windows will install the driver and post the following dialog box.


Update Driver Software - Covaris RF Controller Board
Windows has successfully updated your driver software
Windows has finished installing the driver software for this device:
Covaris RF Controller Board
Close

j. Under **Other devices**, right click device USB Serial Port and select **Update Driver Software.**



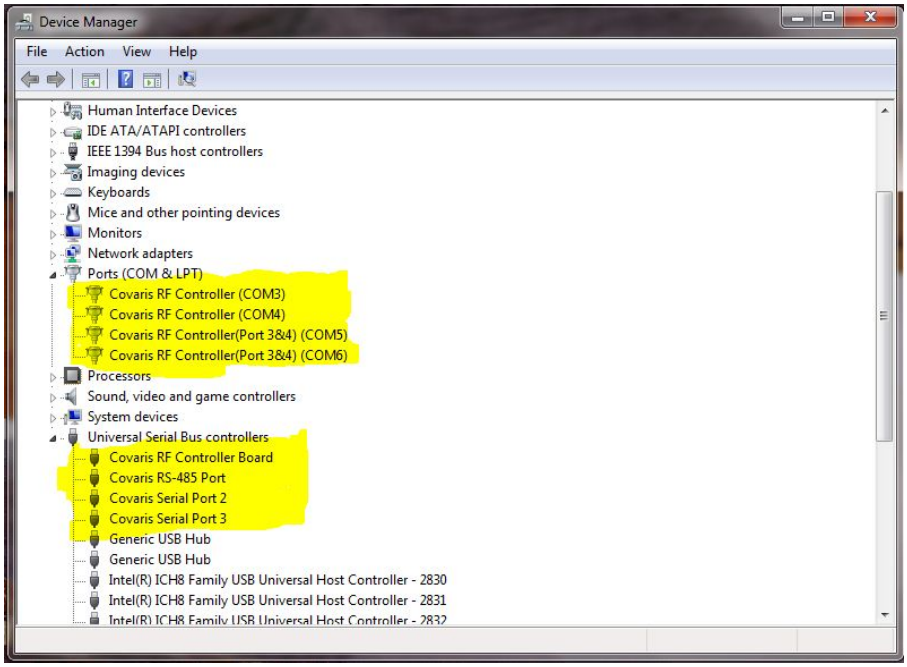
k. Repeat steps E to I three more time for all devices under ‘Other’ devices until they are all gone (should be required 3 times).

l. At the end of the installation, the following devices should be added to Device Manager under Ports(COM & LPT):

- Covaris RF Controller(COM3)
- Covaris RF Controller(COM4)
- Covaris RF Controller(Port 3&4)(COM5)
- Covaris RF Controller(Port 3&4)(COM6)

In addition, 4 devices should be added to Device Manager under Universal Serial Bus Controllers:

- Covaris RF Controller Board
- Covaris RS-485 Port
- Covaris Serial Port 2
- Covaris Serial Port 3



APPENDIX C: Site Preparation and Equipment Installation Requirements

The Covaris E series is delivered in a crate, whose dimensions are approximately 46” wide by 25” deep by 30” high. If doorways or elevators cannot accommodate these dimensions, the instrument and accessories will have to be removed from the crate at the loading dock. If this is necessary, be certain to remove all accessories from the crate before disposal.

Uncrating and Placement of Equipment

Uncrating: Tools required: a #2 Philips screwdriver or power driver. Remove six Philips screws (typically) from the top and remove the top. Remove six screws (typically) from one of the two larger sides, and remove side. Remove the computer, USB cable, chiller hoses, and other accessories from the shipping crate.

The area required for the instrument (see attached drawings) is approximately 24” wide by 30” deep by 19” high (61 cm x 76 cm x 48 cm) and capable of supporting at least 150 pounds. The laptop computer may be placed on top of the E system, or on the bench to the right of the E. If the optional Water Conditioning System (WCS) was ordered, it is placed on the bench to the right of the E, and the laptop may be placed on top of the WCS.

Two people are needed to lift the E from its crate, by placing their hands under the E, inside the foam packing material. A third person, if present, can remove the packing material once the E is lifted, as it often lifts with the instrument. Place the E in the desired location

Two fans maintain a safe operating temperature in the instrument by circulating air over the internal components. Therefore, place the instrument so that there is a minimum of 1” (3cm) between the rear panel and any wall or obstruction for airflow.

The water bath chiller is approximately 10” wide by 18” deep by 22” high (21 cm x 45 cm x 55 cm), and may be placed on the bench to the left of the E, or under the bench. The chiller hoses supplied with the E system are six feet long. If this is not sufficient to reach the chiller, the chiller accessory kit, pn 500102, consisting of six foot extension hoses should be ordered.

Allow sufficient space in front of the bench to access each component of the installation.

Power Requirements

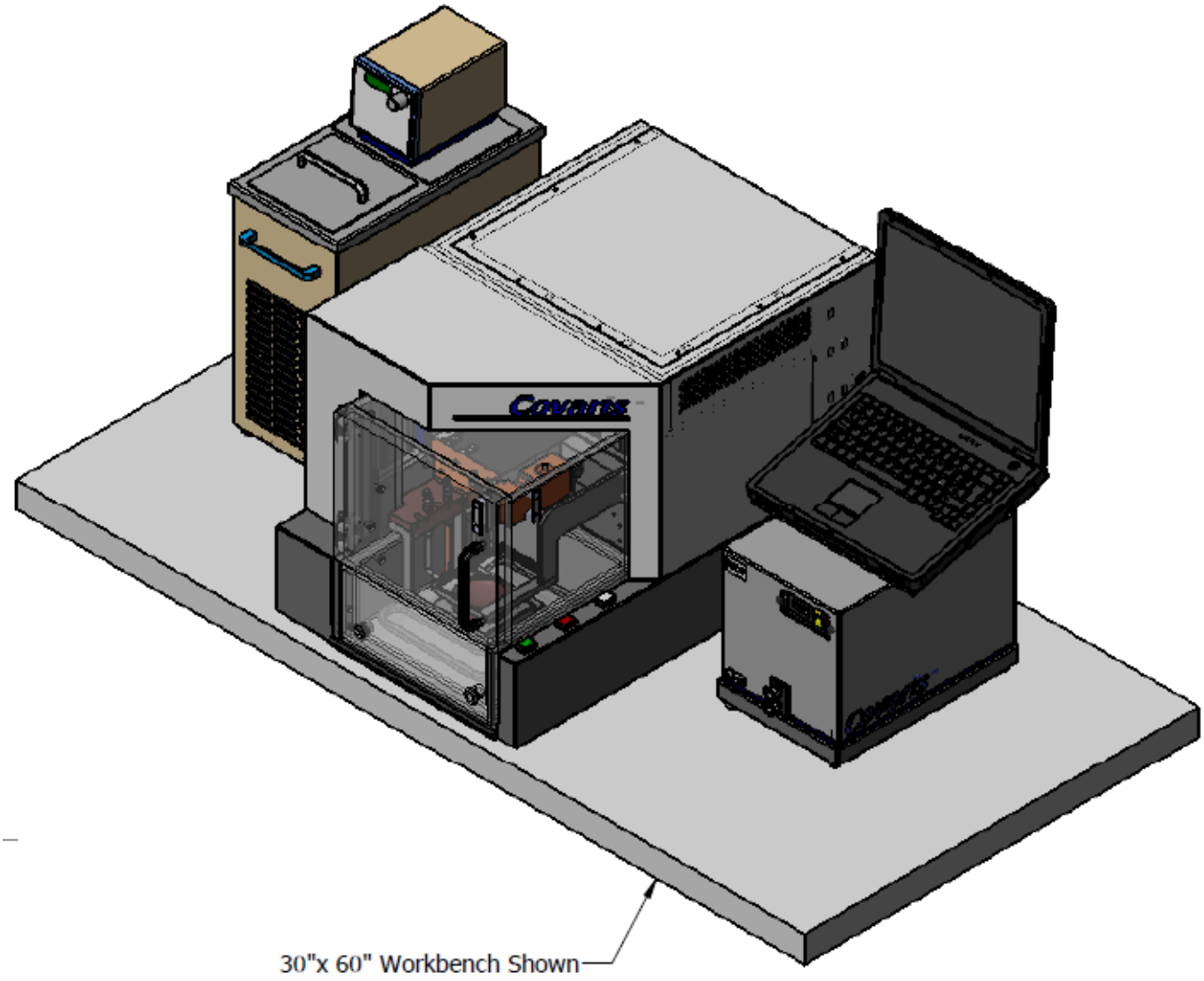
One standard AC outlet each is required for the E system, the computer, the chiller, and the optional Water Conditioning System (WCS), if used.

The instrument draws a maximum of 500 VA. The Covaris E-Series instrument is configured with a universal power supply.

The water bath chiller can draw as much as 12 amps (at 120 VAC). If possible, provide a dedicated circuit for the chiller, separate from the E, the computer, or other electronic equipment. If a dedicated circuit is not feasible, verify that the combination of the chiller and existing loads will not overload the circuit.

The above load ratings are maximum. During typical operation, the E system and all accessories will output less than 1000 watts to the surrounding environment.

WARNING:	**To prevent the possibility of electrical shock, always plug the system into a grounded power source.**


30"x 60" Workbench Shown

APPENDIX D: Humidity and Water Management in Covaris E and LE Systems

Covaris E and LE Systems employ a six-liter tank of distilled or deionized water located inside the instrument housing. This water provides an acoustic path between the system transducer and the vessel in treatment. For the system to operate correctly, the water must be filled to an appropriate level (dependent upon the protocol being run), must be clean, free of algae growth, and degassed to retard bubble formation in the acoustic field outside of the sample vessel. Additionally, bath temperature should be controlled by an external chiller connected to the system's chiller loop. Managing this water and associated humidity is important for the correct operation and reliability of your Covaris system. The following suggestions will help:

- When the system is powered on, the interior is vented by fans located in the rear panel to maintain an appropriate air temperature and humidity within the instrument enclosure. It is recommended, when the acoustic tank is full, that the instrument be left with power ***on***, so that these fans run and retard water condensation on interior surfaces. If it is not practical or desirable to leave these fans running when the instrument is not in use, please leave the front access door ajar so that interior air may exchange with ambient air. The risk of condensate forming within the instrument is increased when operating with a Water Conditioning System (WCS), because the WCS will add a small amount of heat to the bath water.
- To maintain a clean system, Covaris recommends that the bath water be changed daily and that once each month, the tank be disconnected from the WCS (if applicable) and filled with a 10% solution of bleach (e.g. Clorox). Run this solution through the degas system for a few minutes and change back to pure water. The bleach will kill algae growth that may have begun inside hoses or along the inner wall of the tank.
- Employing the optional WCS with an E or LE System precludes the need for a daily change of bath water. It is, however, important that water which evaporates each day be made up with fresh distilled or deionized water. It is also important that a monthly bleaching protocol continue to be followed, as the WCS will retard but cannot completely prevent algae from taking hold in hoses and interior tank surfaces. Detach the WCS water flow from system when bleaching.
- When the system is being employed with a chiller set to a low temperature, you may notice condensate water forming on the black manifold block, on hoses leading to the degas pump, and around the fittings and chassis of the WCS (if employed). Particularly in summer, when relative humidity is higher, condensate may drip onto interior surfaces and small amounts may leak onto the laboratory bench. This condensation is expected and no harm is being done to the system. Excess condensate dripping onto a laboratory bench may be captured by placing an absorbent pad (e.g. VWR 82020-845) beneath the system and/or WCS.
- If operating the acoustic bath at temperatures at or near 4°C, it is important to realize that temperatures in the chiller will be approaching zero degrees. To retard freezing within the chiller, it is recommended that a 20% solution of ethylene glycol be employed in the chiller water. Most chiller and laboratory suppliers carry ethylene glycol for this purpose. Do not employ automotive anti-freeze, and do not put glycol in the E or LE acoustic bath water.

NOTE: The chiller attaches via the quick connect fittings on the left side of cabinet. Do not connect chiller to the fittings on the front of the acrylic water tank.

APPENDIX E: Recommended E220(X) or LE220 IQ / OQ / PQ

All Covaris instruments are rigorously tested at the Manufacturing Facility prior to shipment. This is done to ensure that your Covaris device is of the highest quality and meets your needs for consistent, efficient, and reliable Sample Preparation.

In the event that you require a method for independent verification of your Covaris Instrument, a set of recommended IQ/OQ/PQ activities and requirements are provided in the following Checklist format. If desired, this Checklist can be completed and retained for your personal records. These suggested activities are basic in nature, and intended only for high-level instrument qualification. Given your situation, you may wish to supplement these activities with application-specific performance activities and acceptance criteria.

DEFINITIONS

IQ (Installation Qualification): ensure correct instrument has been delivered and installed in accordance with manufacturer's requirements

OQ (Operation Qualification): ensure instrument is functioning in accordance with specification and all measurement sensors, safety interlocks, and software operate as intended

PQ (Performance Qualification): demonstrate the instrument continues to meet specification appropriate to routine use

E220 / LE220 SERIES - INSTALLATION QUALIFICATION (IQ)

DATE:	INSTRUMENT S/N:	COMPLETED BY:

Action / Requirements	Ref. Section	Requirement Met?
Section 1: Shipping Contents and Instrument Condition		
Instrument is successfully unpacked and all items noted in Packing List are present. Denote any missing items here: _____		☐ YES ☐ NO
There is no damage evident to the instrument or accessories. *If damage is present:* Description of Damage:_____ Corrective Action: _____		☐ YES ☐ NO
Section 2: Verify Proper Site Conditions		
Adequate space and ventilation are present for the instruments, and the room conditions (temp, humidity, etc.) are within recommended limits for the device per the User Manual. *If unacceptable:* Non – Conformity: _____ Corrective Action: _____		☐ YES ☐ NO
Three (3) appropriate power outlets are available for the device, laptop, and accessory Chiller (if necessary).		☐ YES ☐ NO
Section 3: Component Assembly		
Connect the laptop and instrument to line power. The line cords are verified to be in proper condition and match the Wall Outlet. Connect laptop to instrument via USB Cable.		☐ YES ☐ NO
Remove the water tank from instrument and take off plastic packaging. Verify there are no visible cracks or damage. Do not fill tank with water yet, but place back into the instrument.		☐ YES ☐ NO
Connect Chiller to hoses to the instrument quick-connect fittings and fill reservoir for Chiller. Plug in Chiller and turn on (if not already on). Verify the Temperature Set Point can be adjusted and set it below 20C. Verify that the water chills over time.		☐ YES ☐ NO
Power ON instrument with ON/OFF Switch and verify: 1) Instrument Fans are now audible. 2) Internal instrument lights above plate sample holder turn on.		☐ YES ☐ NO

E220 / LE220 SERIES – OPERATIONAL QUALIFICATION (OQ)

(1 of 2)

DATE:	INSTRUMENT S/N:	COMPLETED BY:

Action / Requirements	Ref. Section	Requirement Met?
Section 1: Instrument Connection		
Verify computer is connected to instrument via USB cable provided. Power on the computer and log in (as required). *Note: If Laptop is not provided by Covaris, refer to SonoLab / Driver installation sections of User Manual.*		☐ YES ☐ NO
Double-click the 'SonoLab' shortcut on the computer desktop; verify the application starts and software requests a 'HOMING' sequence be performed. Follow instructions to perform 'HOMING' and ensure the motion system moves into the LOAD position.		☐ YES ☐ NO
After motion initialization is completed, verify the SonoLab software recognizes the instrument and indicates the instrument type at the top right of the screen.		☐ YES ☐ NO
Section 2: Sensor / Safety Interlock		
Verify Temperature is displayed in SonoLab and is approximately the same as the ambient temperature (since tank is empty). Bath Temp Reading: _____ °C		☐ YES ☐ NO
With transducer in position in empty tank, verify *Low Water Level* indicator bar is lit up RED on SonoLab screen.		☐ YES ☐ NO
Press and hold Green 'DOOR' button at the front of the E220 instrument and open treatment door. Verify that the *Interlock Open* indicator in SonoLab is also now lit up red. Leave the door open and click 'Load Plate' button on the main menu. Verify SonoLab does not allow this command.		☐ YES ☐ NO
Shut door and verify *Interlock Open* indicator is no longer lit up. Click 'Service Bath' button. When transducer and sample mechanical assemblies are above tank, open the instrument door and pull water tank back from instrument a few inches (or completely). Ensure the *Interlock Open* and *Bath Fault or Side Door Fault* SonoLab indicator bars are both lit RED.		☐ YES ☐ NO
Fill Water Bath with DI or Distilled water between 10 and 15 marks on left-hand FILL Side of water level label and install filled water tank back into instrument.		☐ YES ☐ NO

E220 / LE220 SERIES – OPERATIONAL QUALIFICATION (OQ)

(2 of 2)

DATE:	INSTRUMENT S/N:	COMPLETED BY:

Section 3: Degas / Run Method		
Click 'LOAD PLATE' button in SonoLab and verify transducer submerges. Click 'DEGAS ON' and ensure degas pump can audibly be heard running and bubbles are exiting the outlet tube.		☐ YES ☐ NO
<u>Acoustic Method Verification</u> ➢ **Install an appropriate-sized rack populated with sample tubes into the plate holder.** ➢ **Create a new method by clicking the SonoLab *Method Editor*.** ➢ **Create settings for the method which treats one or more of the populated tubes with settings indicated:** • Treatment Time = 30 Seconds, Duty Factor = 5.0%, Peak Incident Power = 100 W, Cycles/Burst = 50 ➢ **Click '*SAVE METHOD AS*' and enter "Method Test"; navigate back to Main SonoLab screen.**		☐ YES ☐ NO

Click 'START' on the SonoLab Main Screen to start the recently created Method. Verify the Treatment runs on the correct tube(s) and reasonable Power Levels are displayed by SonoLab.		☐ YES ☐ NO
Before the Treatment ends, press and hold the manual RED 'STOP' button on the front of the instrument (below GREEN 'DOOR' button); ensure the method stops. Click 'ABORT' when prompted.		☐ YES ☐ NO
Back in the SonoLab main screen, click 'START' once again. Before the Treatment ends, click the red STOP button on the SonoLab Screen and verify the Method is suspended. Click Resume when prompted and verify the Method continues where it left off.		☐ YES ☐ NO
Verify that the method completes without any errors and generates a 'PROCESSING COMPLETED' dialog box.		☐ YES ☐ NO

COMMENTS:

E220 / LE220 SERIES – PERFORMANCE QUALIFICIATION (PQ)

(1 of 2)

DATE:	INSTRUMENT S/N:	COMPLETED BY:

Action / Requirements	Ref. Section	Requirement Met?
Power Verification Test		
The following can be performed during observation of normal sample processing or as a stand-alone experiment.* ➢ **Denote rack and tube type used for power verification test. *Rack:* _____ *Tube Type:* _____ ➢ **Install desired sample and medium in at least one tube (or simply fill > 50% with water). Load a method commonly used with the rack and tube types selected. Denote the Peak Incident Power (PIP) and Duty Factors employed for this Method.** *Method Name:* _____ *Peak Incident Power:* _____ *Watts* *Duty Factor :*_____ *%* ➢ **Calculate the 'Expected Power' value by the following:** *Expected Power* = Peak Incident Power X Duty Factor / 100 *Expected Power =* _____ *Watts* ➢ **Per standard practice, allow bath water to Degas at least 45 minutes and configure Chiller to desired setting. For most accurate results, it is recommended that the bath temperature be between 6 and 17C.** ➢ **Run the method. Allow treatment to run for ~ 10 seconds. Monitor the power reading displayed in SonoLab and record the average value below. Verify that turbulence is seen in the water around the tube.** **SonoLab Avg. Power** [ ] *Watts* ➢ **<u>Verify the recorded SonoLab average power does not vary more than 25% from the 'Expected Power' value calculated above.</u>** *Note:* If the Expected Power value is not within specification, repeat test on a different tube and ensure tube does not contain cracks, pits, or other damage. If SonoLab power is still not within specification, contact Covaris Customer Service for support.		*('SonoLab Power' varies from 'Expected Power' less than 25%)* ☐ YES ☐ NO

E220 / LE220 SERIES – PERFORMANCE QUALIFICIATION (PQ)

(2 of 2)

DATE:	INSTRUMENT S/N:	COMPLETED BY:

Degas Performance Test – OPTIONAL ***(Calibrated O2 Meter Required)***		
➢ **Fill Water Bath with fresh water (10-15 on 'Fill' side of Water Level Label). Use a calibrated Dissolved Oxygen Meter (i.e. HACH HQ Series) to measure the baseline oxygen level.** Baseline Dissolved Oxygen Level : _____ PPM (mg/L) ➢ **Turn Chiller OFF (or disconnect from instrument).** ➢ **Install water bath in device and turn on Degas Pump using SonoLab controls.** ➢ **Allow Pump to run for 1 hour.** ➢ **Use the 02 Meter to measure the water bath Oxygen content. Verify the value is LESS THAN 3.5 PPM** Degas Dissolved Oxygen Level : _____ PPM (mg/L)		☐ YES ☐ NO
User-Defined Test (As Required)		
		☐ YES ☐ NO

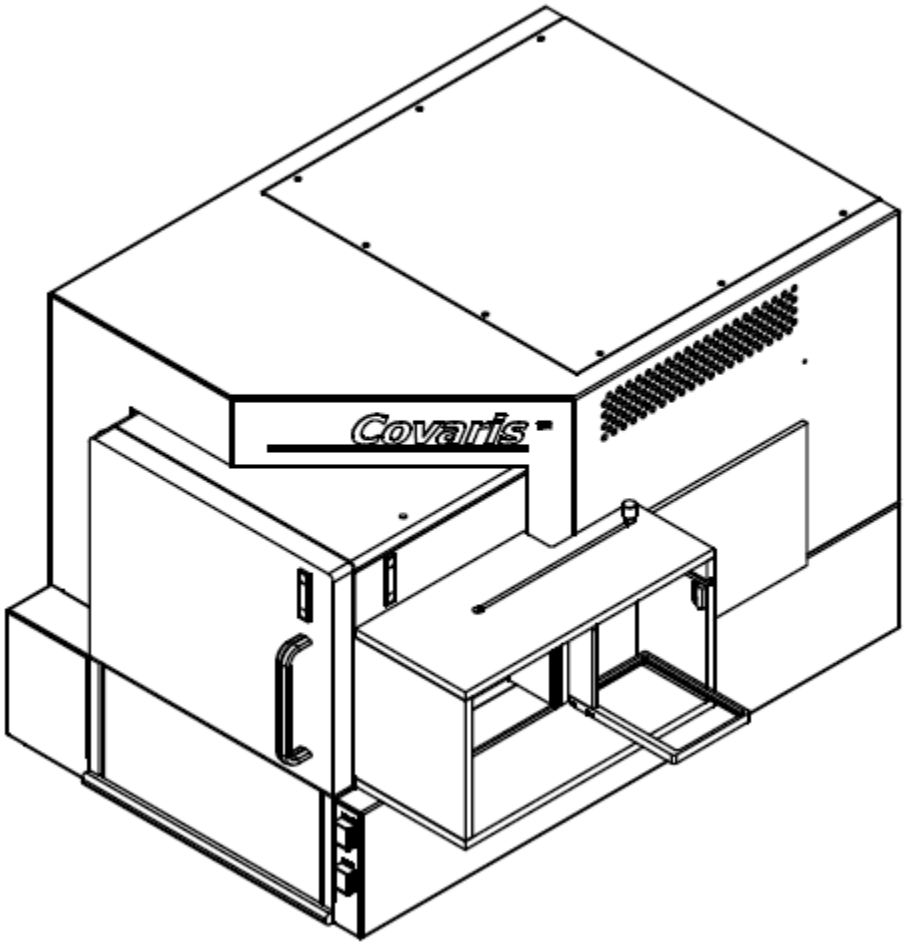
COMMENTS:

APPENDIX F: "R" version of E220 and LE220 (E220R and LE220R)

"R" versions of the E220 and LE220 instruments operate identically to their non-R models, but are equipped with a side-access door and controllable swing arm to allow a robotic system to place a rack or plate of samples on the holder frame of the instrument. The system may be controlled via an installed set of remote control commands contained in an Applications Programming Interface dynamic link library.

Magnetic sensors are employed on the side-access door and acrylic housing. These sensors must detect a "door closed" or "work cell present" status before ultrasonic processing will be allowed in the instrument.

For further information on integrating an R version instrument with an automated handling system, please contact Covaris Customer Service.



Outline of an R version E or LE instrument, illustrating the side access door and swing arm